REMARKS

Claims 2 and11-30 have been canceled without prejudice . Claims 1 and 3-10 remain before the Examiner for reconsideration.

In the Advisory Office Action, the Examiner maintained the rejection Claims 1-10 under 35 U.S.C. Section 102 (a or b) as being anticipated by Havens et al. Specifically, the Examiner further asserted that:

> Applicants recognize that Havens et al obtain a maximum of 5mg protein/g polymer which is 0.5 wt% of protein contained by the polymer. However, applicants urge that not all protein is enzyme, and that the concentration of enzyme contained by the polymer is much lower and enzyme loading is probably less that 0.wt%. However, Havens et al disclose partial purification of the enzyme using 20-40% ammonium sulfate (page 2256, left column, first full paragraph). Due to this purification with ammonium sulfate, it appears that Havens et al obtain an enzyme loading of greater than approximately 0.1 percent by weight. Of the 5 mg protein present, it appears that at least 1 mg would be enzyme due to the ammonium sulfate purification. It should be noted that the claims require greater than approximately 0.1% enzyme. The term "approximately" permits an amount of enzyme loading lower than 0.1%.

Applicants respectfully traverse the Examiner's rejection.

Once again, Applicants are not claiming merely the addition of surfactant during the synthesis of a polymer immobilizing an enzyme, but including a sufficient amount of a surfactant in the reaction mixture to increase enzyme activity at an enzyme loading than is substantially higher than the enzyme loadings disclosed in Havens et al. In the Amendment filed September 16, 2002, Applicants amended Claim 1 to set forth an enzyme loading of at least 0.1 weight percent. Including a sufficient amount of a surfactant in the reaction mixture to increase enzyme activity at any enzyme loading, let alone the relatively high enzyme loading of the present invention, is not disclosed or suggested in Havens et al.

In Havens et al., a very crude (impure) protein preparation was used at a maximum concentration of 5mg/g polymer. Although this is 0.5 wt% of protein preparation, the concentration of enzyme in that preparation is much lower. Although not indicated, the enzyme loading in the polymer of Havens et al. is quite low. Indeed, the low enzyme loading of Havens et al. can be demonstrated mathematically. Declaration of Keith E. LeJeune, paragraph 5.

In that regard, the turnover number (or kcat) for the subject enzyme of Havens et al. (Parathion hydrolase from P. diminuta) on parathion is well established as 1,067 umol/min/mg enzyme. (See, for example, Dumas et al., 1989 J. Biol. Chem. 264, 19659-19665 and Dumas et al., 1990 Arch. of Biochem. Biophys., 277, 1, 155-159). The inventors of the present application have verified these findings in the laboratory. Declaration of Keith E. LeJeune, paragraph 6.

In Havens et al, the polymer used to initiate the hydrolysis (shown in Figure 1) is described as a 5.5g polymer carrying 2.9 mg protein / g prepolymer, or approximately 16mg of protein. Havens et al. also describes that the standard assay was to apply substrate solutions to the polymers at 10-times the polymer mass, or in that case 55ml of parathion solution. Figure 1 shows a reduction of parathion concentration from 0.045 to 0.01 nmol/uL in 5 minutes, or a rate of 0.007 nmol/uL/min (or also 7 nmol/mL/min). Multiplying by the volume of 55 ml, one calculates the apparent reaction rate to be 385 nmol/min. Considering 16 mg of the protein prep was required to achieve this rate, the actual observed rate was 24 nmol/min/mg protein. Declaration of Keith E. LeJeune, paragraph 7.

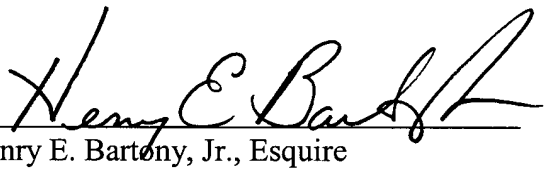
If one conservatively assumes a low activity retention of enzyme of 1% during polymerization, the resulting rate in the Havens et al. experiment should have been 10.67 umol/min/mg enzyme, if the protein preparation of Havens et al. was pure enzyme. The division of their achieved rate (24 nmol/min/mg protein) by that expected (10.67 umol/min/mg enzyme) for pure enzyme, provides the purity of the prep (0.024/10.67) or 0.002 mg enzyme/ mg protein. This calculation indicates that the protocols described by Havens et al. employed a protein prep that was less than or equal to 0.2% pure enzyme.

Enzyme loading would thus be less than or equal to 0.0058 mg enzyme/ g prepolymer ([0.002 mg enzyme / mg protein] * [2.9 mg protein / g prepolymer]) or .00058 wt percent enzyme loading (0.0058 mg enyme/1000mg prepolymer * 100). Even assuming an extremely low activity retention of 0.1%, the wt percent enzyme loading of the polymer of Havens et al. would be only .0058 wt%, well below the 0.1 wt% enzyme loading of the present invention. Declaration of Keith E. LeJeune, paragraph 8.

In light of the above remarks, Applicants respectfully request that the Examiner withdraw his rejection of Claims 1 and 3-10, and that the Examiner indicate the allowability of Claims 1 and 3-10 and arrange for an official Notice of Allowance to be issued in due course.

Respectfully submitted,

AGENTASE, LLC..

By 

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